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(54) PREPARATION OF PROTEIN MICROSPHERES, FILMS AND COATINGS

HERSTELLUNG VON PROTEINMIKROSPÄHREN, FILMEN UND BESCHICHTUNGEN

PREPARATION DE MICROSPHERES, DE PELLICULES ET DE REVETEMENTS A PARTIR DE PROTEINES

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Description

[0001] The invention relates to microphores, films and coatings made from proteins or modified proteins and in particular to improvements in the methods of preparation thereof which result in more stable products. The microphores, films and coatings produced in accordance with the invention are suitable for a variety of biomedical applications.

[0002] The term "microphores" is generally employed to describe colloidal particles which are substantially spherical and have a diameter in the range 10 nm to 2 mm. Particles having a diameter of less than 1μ are sometimes called "nanoparticles". Microspheres made from a very wide range of natural and synthetic polymers have found use in a variety of biomedical applications. They can be labelled with markers (labels or sensing devices) and transported through various media both in-vitro and in-vivo. The labels may be chemical fluorescent, magnetic or radioactive and thus they may, by appropriate sensing equipment, be observed when in use. The sort of applications for which microspheres have been used are diagnostic screening, cell separation, immunoassays, studies of phagocytosis and blood flow, studies of cell motility, haemoperfusion and extracorporeal therapy, drug delivery devices, targeted drug delivery, cell encapsulation and endovasular embolisation.

[0003] An important property which microspheres must possess for biomedical applications is biocompatibility. They should be as resistant as possible to attack from the immune system in-vivo. Further, for many applications it is important that the microspheres be biodegradable and/or resorbable in the body once their function has been discharged. Also, in other cases, they should be small enough for easy introduction into the body.

[0004] For these reasons, naturally occurring polymer materials such as proteins have been the subject of much study for the preparation of microspheres. Nanoparticles as small as 100 nm can be prepared from, for example, albumin using certain preparation techniques and this is very useful for, among other things, injectable preparations.

[0005] Because of their biocompatability some proteins have been used in making coatings for artificial prostheses which will be introduced into the human body and therefore in contact with body fluids. As with microspheres any such coating should be as resistant as possible to attack from the immune system and furthermore should not be thrombolytic i.e. should cause only minimal platelet activation.

[0006] It is known that the blood biocompatibility of arterial prostheses is improved by coating the surfaces with albumin as demonstrated by Kottke-Marchant et al in Biomaterials 1989 10 147-155. Indeed albumin has been a particular material of choice for both coatings and microspheres because it is non-antigenic, biodegradable and readily available.

[0007] A number of methods are known for preparing protein microspheres and films and protein coatings for prostheses but certain drawbacks are associated with them all.

[0008] For example, a well-known method of preparing protein microspheres is suspension cross-linking. In this process an aqueous solution of protein is added to an immiscible liquid or oil phase. Droplets of protein are dispersed by high speed stirring and then hardening or stabilization of the droplets to form microspheres is brought about by heating of the suspension to, for example, a temperature above 80°C or alternatively by chemical cross-linking employing a cross-linking agent such as glutaraldehyde. Various methods of preparation of albumin microspheres by the suspension cross-linking technique are described by Arshady in Journal of Controlled Release, 14 (1990) 111-131.

[0009] A disadvantage of preparing microspheres by the suspension cross-linking technique is that it is difficult to produce microspheres less than 500 nm in size, although nanospheres of about 100 nm diameter have been prepared using high power ultra-sonication. A further disadvantage is that the cross-linking agents used are often toxic which is not conducive to biocompatability.

[0010] Apart from suspension cross-linking protein microspheres, especially gelatin microspheres, have been prepared by coacervation or controlled desolvation. This procedure has also been used to prepare albumin microspheres in the size range 0.1 to 5μm by Knop, et al (1975) using ethanol as the coacervation agent added to an aqueous solution of albumin. Ishizak et al (1985) have prepared albumin microspheres in the size range 0.5 to 1.5μm using isopropyl alcohol as the coacervation agent. A similar technique has been developed to prepare 200-500 nm nanoparticles by adding acetone to an aqueous solution of human serum albumin and heating the colloidal system (Chen et al, 1994, J. Microencapsulation, 11, 395-407). In an alternative approach, chemical crosslinking agents such as gluteraldehyde have been used to harden the microspheres (Lin et al (1993) J. Drug Targeting 1 pp 237-243).

[0011] Coacervation methods for preparing protein microspheres are simpler to perform than suspension cross-linking methods and the particles are less toxic. A disadvantage however is that the particles are not particularly stable and aggregate easily to form larger microspheres. It has been difficult, to date, to use coacervation methods to prepare protein nanospheres around 200 nm or less which may have potential use as injectable preparations.

[0012] Albumin microparticles (2-10 μm) have also been prepared by spray drying followed by heat stabilisation at 100°C or 150°C for 6-24 hours (Pavanetto et al., J. Microencapsulation, 11, 445-454). The main advantage of spray drying is that albumin microparticles are free of oil residues or organic solvent and the process is useful for continuous operation.

[0013] The problems of toxicity of cross-linking agents and of stability also arise in the case of protein coatings to

be applied to artificial prostheses. In particular, in the physiological environment the coating is subject to wear leaving areas of the prosthesis material exposed which may lead to an unwanted immune response or clot formation.

[0014] There is thus a continuing need for improved methods of preparation of protein microspheres, films and coatings which do not have the various disadvantages out-lined above. The present inventors have developed new methods of preparing protein microspheres, films and coatings which meet this goal and all the methods are based on the simple observation that when the pH of an aqueous albumin solution is lowered to about 4.4 to 4.7 with an α-hydroxy acid, e. g. lactic acid, a rapid and extensive precipitate of albumen forms and this precipitate is unusually stable. The same effect is observed with other proteins although the pH range at which precipitation occurs varies.

[0015] Thus in its first aspect the invention provides a process for stabilizing a microsphere, film or coating made from at least one protein or modified protein which comprises preparing said protein microsphere, film or coating in the presence of an aqueous solution of at least one α-hydroxy acid or a derivative or analogue thereof.

[0016] It is to be understood that herein the term protein is intended to include peptides, polypeptides, metalloproteins, glycoproteins and lipoproteins and the term "modified protein" refers to proteins modified so as to have an additional molecule attached thereto, that would not naturally be associated with the protein. For example, the modified protein might consist of the protein conjugated to another organic polymer such as polyethylene glycol, polylactide or other polymer which can influence the surface characteristics of the microsphere, film or coating advantageously from a biocompatability point of view.

[0017] Preferred proteins which may be used in the process of the invention are albumen, gelatin, zein, casein, collagen or fibrinogen. Particularly preferred is albumen, either human serum albumen or ovalbumen.

[0018] Preferred α-hydroxy acids for use in the invention are glycolic acid, lactic acid, hydroxybutyric acid or mixtures of two or more thereof. Particularly preferred is lactic acid. By α-hydroxy acid derivative is meant an α-hydroxy acid derivatised by conjugation to another molecule, for example, polyethylene glycol, which may have a beneficial effect on the surface characteristics of the microspheres, films or coatings made by the process of the present invention.

[0019] Known processes as described above for making microspheres may be modified by combining with the stabilization method of the present invention to provide new and improved methods and products.

[0020] For example, a method of making a microsphere in accordance with one embodiment of the invention comprises the steps of:-

(a) mixing an aqueous solution of at least one protein or modified protein with an aqueous solution of at least one α-hydroxy acid or analogue or derivative thereof,

(b) adding to the mixture prepared in step (a) a coacervation agent which results in the formation of mirospheres incorporating said protein or modified protein,

(c) evaporating said coacervation agent and

(d) recovering the microspheres from the aqueous solution.

[0021] As coacervation agent it is preferable to use acetone, ethanol or isopropanol and most preferably acetone. Its addition results in controlled desolvation of the microspheres which may then be recovered from the aqueous solution by centrifugation, sonication or filtration.

[0022] In the coacervation method described above a suitable protein concentration is about 0.1 to 10% by weight and the volume ratio of coacervation agent to protein solution is preferably about 2-3:1.

[0023] A particular advantage of the coacervation method described above is that the microspheres produced are nanoparticles e.g. about 200 nm. They are therefore suitable for making injectable preparations. It is possible to include in the nanoparticles during their preparation a pharmaceutically active agent for example or a material detectable by a bio-imaging procedure so that they may be detected in-vivo. This is achieved by including the active agent or bio-imaging material in the aqueous mixture of protein and α-hydroxy acid before the coacervation agent is added. Microspheres so produced can then be mixed with a pharmaceutically acceptable carrier or diluent to form a pharmaceutical composition or preparation suitable for bio-imaging. As aforesaid albumin, especially human serum albumin is particularly preferred for the preparation of microspheres for biomedical applications and lactic acid is the preferred stabilizer.

[0024] A method of making a microsphere in accordance with another embodiment of the invention comprises the steps of:-

(a) mixing an aqueous solution of at least one protein or modified protein with an aqueous solution of at least one α-hydroxy acid or analogue or derivative thereof,

(b) adding the mixture prepared in step (a) to a water-immiscible oil,

(c) stirring the mixture prepared in step (b) to form microspheres, and

(d) recovering the microphores from the water-immiscible oil.

[0025] Formation of microspheres in step (c) is enhanced if the stirring is carried out at an elevated temperature, for example at about 37 to about 50°C. Preferably, prior to the recovery step (d), a solvent is added to the microspheres prepared in step (c) to aid dispersion.

[0026] Microspheres produced by the above described emulsification method are usually in the 10 to 50 μm size range. The method allows the microspheres to be hardened without the addition of a toxic cross-linking agent which is a particular advantage. The protein solution is preferably more concentrated than with the coacervation method, about 10 to 30% by weight being particularly suitable. Preferably, the volume ratio of oil to aqueous phase is about 100.1. As the solvent for aiding microsphere dispersion, acetone or ethyl acetate can be used. A particularly preferred water-immiscible oil is soya oil. Albumin is the preferred protein with lactic acid as the stabilizer.

[0027] As with the coacervation method it is possible to add a pharmaceutically active agent or bio-imaging material to the aqueous phase before mixing with the oil and so produce microspheres suitable for use in pharmaceutical compositions or bio-imaging preparations.

[0028] Other known uses of microspheres in general include drug targeting and in-vivo diagnosis where it is desired to transport an active agent or detectable agent respectively to a particular site in the body. Microspheres made by both the coacervation and emulsification methods of the invention can be used in this way by attaching to the surface of a suitably loaded microsphere a molecule recognised by and having a particular affinity for a cellular receptor in the human or animal body. It is also possible to incorporate an antigenic material into the microspheres so that they can be mixed with a suitable pharmaceutical carrier and used as a vaccine.

[0029] In yet another aspect the invention provides a method of making a film from a protein or modified protein which comprises the particular steps of:-

(a) cooling to approximately 4°C an aqueous solution of said protein or modified protein,

(b) mixing the cooled solution prepared in step (a) with an aqueous solution of said α-hydroxy acid or an analogue or derivative thereof,

(c) spreading the solution prepared in step (b) as a thin layer over a solid surface and

(d) drying said thin layer to form a film.

Preferably the drying stage is carried out at between 50 and 70°C.

[0030] Albumin films made by the method of the invention have been shown to be much more resistant to dissolution in water than films made in the absence of the α-hydroxy acid.

[0031] Finally, in yet another aspect the invention provides a process for coating an article with a protein or modified protein layer which comprises dipping said article or a portion thereof into an aqueous solution comprising said protein or modified protein and an α-hydroxy acid.

[0032] In the alternative a previously protein-coated article may simply be dipped into the α-hydroxy acid solution.

[0033] In particularly preferred embodiments of all the methods described herein the protein and/or the α-hydroxy acid is modified or derivatised by attachment of another polymer which is known to influence the surface characteristics of the microsphere, film or coating produced. For example polyethylene glycol is known to have a beneficial effect on the surface of microspheres by rendering them more hydrophilic and thus resistant to sequestration by cells of the reticulo-endothelial system. Thus the methods of the invention may be carried out using an α-hydroxy acid or protein conjugated to polyethylene glycol or other suitable polymer, for example polylactide.

[0034] The invention will now be described with reference to the following non-limiting Examples:-

Example 1

Preparation of Albumin Nanoparticles by the Coacervation Method

[0035] An aqueous albumin solution (ovalbumin or human serum albumin) was prepared at a concentration of 2% by weight and 2 mls of albumin solution were mixed with 20 μl lactic acid (LA) (F.W. 90.8; 85+% solution in water). 5.2 mls of acetone were then added and the mixture stirred overnight with a magnetic stirrer to evaporate the acetone. Nanoparticles were recovered from the aqueous solution by centrifuging and sonication and then resuspended in distilled water.

[0036] The size and Zeta potential of microspheres prepared as described above were measured and are shown in Table 1 below.

Nanoparticles	Particle size (nm)	Zeta potential (mV)
LA stabilised HSA	200	-11.9 ± 0.9
Glutaraldehyde stabilised HSA	140	-19.1 ± 0.7

[0037] A marked reduction in Zeta potential of lactic acid stabilized nanoparticles relative to glutaraldehyde stabilised nanoparticles was evident indicating differences in surface chemistry. The glutaraldehyde stabilised nanoparticles were prepared as described in Lin et al referred to above.

[0038] The nanoparticles stablised with lactic acid as described above have a particle size of 200 nm which renders them suitable for pharmaceutical use, particularly for injectable preparations. They are expected to be less toxic than albumin nanoparticles produced using glutaraldehyde cross-linking.

[0039] Nanoparticle suspensions appear to be stable over 7 days in water at room temperature. Ovalbumin precipitates are formed when the pH of ovalbumin solutions at 37°C is adjusted to between 4.4 and 4.7 with lactic acid. These precipitates resist breakdown by 2% SDS solution when exposed for 72 hours at room temperature. A large component of the precipitate appears to be resistant to similar treatment in 5% SDS.

Example 2

Preparation of Albumin Microspheres by the Emulsification Method

[0040] 100 ml of soya oil containing 2% of Span 85 as a surfactant was stirred in an ice bath at 2000 rpm using an overhead stirrer. 1.0 ml of a 20% (w/v) aqueous solution of ovalbumin (OVA) was mixed with 100 µl of lactic acid and added dropwise to the soya oil which was stirred continuously. The resulting emulsion was stirred for 10 minutes and then for a further 30 minutes at 45°C. 50 ml of acetone or ethyl acetate (as a dispersion aid) was added and the suspension of hardened OVA microspheres was sonicated for 10 minutes. The OVA microspheres were separated by centrifuging and washed with acetone or ethyl acetate to remove residual oil.

[0041] Measurement of the microsphere size by laser light scattering revealed the diameter to be in the 10 to 50 µm range with the mean size around 25µm.

[0042] Simple dissolution studies carried out in distilled water under ambient conditions revealed that the OVA microparticles prepared using lactic acid appeared to be physically stable for at least 3-5 days. In contrast, OVA microparticles produced using the above technique without lactic acid dissolved in less than 10 minutes.

Example 3

Preparation of Ovalbumin Film

[0043] 50 µl of lactic acid were added to 12 ml of a 2% solution of ovalbumin which had been cooled in an ice bath. 4 ml of the mixture were added to each of three 50 ml beakers and retained at 60°C and 32°C overnight and at room temperature for 16 hours respectively to obtain dried OVA films.

[0044] Films of ovalbumin were also produced by the above method but without addition of lactic acid.

[0045] A dissolution experiment was performed by adding 20 ml of distilled water to each beaker and measuring the concentration of free albumin in solution over time at room temperature. This was achieved by measuring the absorbance of the dissolution medium at 280 nm using a UV spectrophotometer and comparing this with a calibration curve constructed from a series dilution of ovalbumin in water. The percentage film dissolution is shown in Table 2 as a function of time.

Table 2

OVA film dissolution vs time at room temperature.								
	% Film dissolution							
Time (hours)		0.25	1	4	6	24	48	96
OVA	RT*	92.5	94.4					

* Film drying temperature

Table 2 (continued)

OVA film dissolution vs time at room temperature.								
	% Film dissolution							
Time (hours)		0.25	1	4	6	24	48	96
OVA/LA		42.5	100.0					
OVA	32	87.5	100.0					
OVA/LA		47.5	100.0					
OVA	60	77.5	94.4					
OVA/LA		25.0	38.9	36.1	38.9	38.9	41.7	41.7

[0046] The data presented in Table 2 reveal that greater film stability is apparent when lactic acid is added to OVA solutions before drying. A marked improvement is obtained when OVA films containing lactic acid are dried at 60°C.

[0047] The film forming technique described above is particularly useful for coating medical textiles such as those used for arterial grafts and soft tissue repair so as to decrease permeability and improve biocompatibility.

Claims

1. A process for stabilizing a microsphere, film or coating made from at least one water soluble protein or modified protein, which process comprises preparing said protein microsphere, film or coating in the presence of an aqueous solution of at least one α-hydroxy acid or a derivative or analogue thereof.

2. A process as claimed in claim 1 wherein said α-hydroxy acid is glycolic acid, lactic acid, α-hydroxybutyric acid or a mixture of two or more thereof.

3. A process as claimed in claim 1 wherein said derivative of an α-hydroxy acid is an α-hydroxy acid conjugated to polyethylene glycol.

4. A process as claimed in claim 2 or claim 3 wherein said α-hydroxy acid is lactic acid.

5. A process as claimed in any preceding claim wherein said protein is albumen, gelatin, collagen, zein, casein or fibrinogen.

6. A process as claimed in claim 5 wherein said protein is human serum albumin.

7. A process as claimed in claim 5 wherein said protein is ovalbumin.

8. A process as claimed in any preceding claim wherein said protein is modified by having another organic polymer attached thereto.

9. A process as claimed in any preceding claim which comprises the particular steps of:-

   (a) mixing an aqueous solution of at least one protein or modified protein with an aqueous solution of at least one α-hydroxy acid or analogue or derivative thereof,

   (b) adding to the mixture prepared in step (a) a coacervation agent which results in the formation of microspheres incorporating said protein or modified protein,

   (c) evaporating said coacervation agent and

   (d) recovering the microspheres from the aqueous solution.

10. A process as claimed in claim 9 wherein said coacervation agent is selected from acetone, ethanol and isopropanol.

11. A process as claimed in claim 9 or claim 10 wherein prior to addition of the coacervation agent a pharmaceutically active agent is added to the mixture prepared in step (a).

12. A process as claimed in claim 9 or claim 10 wherein prior to the addition of the coacervation agent a material detectable by a bio-imaging procedure is added to the mixture prepared in step (a).

13. A process as claimed in any one of claims 1 to 8 comprising the particular steps of:-

(a) mixing an aqueous solution of at least one protein or modified protein with an aqueous solution of at least one α-hydroxy acid or analogue or derivative thereof,

(b) adding the mixture prepared in step (a) to a water-immiscible oil,

(c) stirring the mixture prepared in step (b) to form microspheres,

(d) recovering the microspheres from the water-immiscible oil.

14. A process as claimed in claim 13 wherein prior to recovery from the water-immicible oil a solvent is added to the microspheres prepared in step (c) to aid dispersion.

15. A process as claimed in claim 14 wherein the solvent added to aid dispersion is acetone or ethyl acetate.

16. A process as claimed in any one of claims 13 to 15 wherein the microspheres are sonicated prior to recovery from the water-immiscible oil.

17. A process as claimed in any one of claims 13 to 16 wherein a pharmaceutically active agent is added to the mixture prepared in step (a) prior to mixing with the water-immiscible oil.

18. A process as claimed in any one of claims 13 to 16 wherein a material detectable by a bio-imaging procedure is added to the mixture prepared in step (a) prior to mixing with the water immiscible oil.

19. A process as claimed in any one of claims 9 to 18 which comprises the additional step of attaching to the surface of the microspheres so formed a molecule recognised by and having an affinity for a cellular receptor in the human or animal body.

20. A process as claimed in any one of claims 9 to 18 which comprises the additional step of attaching to the surface of the microspheres so formed an antigenic material.

21. A microsphere prepared by the process of any one of claims 9 to 20.

22. A pharmaceutical composition which comprises a plurality of microspheres prepared by the process of claim 11 or claim 17 and a pharmaceutically acceptable carrier or diluent.

23. A vaccine which comprises a plurality of microspheres prepared by the process of claim 19 and a pharmaceutically acceptable carrier or diluent.

24. A bio-imaging preparation which comprises a plurality of microspheres prepared by the process of claim 12 or claim 18 and a pharmaceutically acceptable carrier or diluent.

25. A process as claimed in any one of claims 1 to 8 which comprises the particular steps of:-

(a) cooling to approximately 4°C an aqueous solution of said protein or modified protein,

(b) mixing the cooled solution prepared in step (a) with an aqueous solution of said α-hydroxy acid or an analogue or derivative thereof,

(c) spreading the solution prepared in step (b) as a thin layer over a solid surface and

(d) drying said thin layer to form a film.

26. A process as claimed in claim 25 wherein said layer of solution is dried at between 50 and 70°C.

27. A film made by the process of claims 25 or 26.

28. A process for coating an article with a protein or modified protein layer which comprises dipping said article or a portion thereof into an aqueous solution comprising said protein or modified protein and an α-hydroxy acid or analogue or derivative thereof.

29. A process for treating an article coated with a protein or modified protein which comprises dipping said article or a portion thereof into an aqueous solution comprising an α-hydroxy acid or analogue or derivative thereof.

30. A process as claimed in claim 28 having the features of any one of claims 2 to 8.

Patentansprüche

1. Verfahren zur Stabilisierung einer Mikrosphäre, eines Films oder einer Beschichtung, die aus mindestens einem wasserlöslichen Protein oder modifizierten Protein hergestellt sind, wobei das Verfahren das Herstellen der Proteinmikrosphäre, des Proteinfilms oder der Proteinbeschichtung in Gegenwart einer wäßrigen Lösung von mindestens einer α-Hydroxysäure oder eines Derivats oder Analogons davon umfaßt.

2. Verfahren nach Anspruch 1, worin die α-Hydroxysäure Glykolsäure, Milchsäure, α-Hydroxybuttersäure oder ein Gemisch von zwei oder mehr davon ist.

3. Verfahren nach Anspruch 1, worin das Derivat einer α-Hydroxysäure eine α-Hydroxysäure konjugiert an Polyethylenglykol ist.

4. Verfahren nach Anspruch 2 oder 3, worin die α-Hydroxysäure Milchsäure ist.

5. Verfahren nach einem der vorhergehenden Ansprüche, worin das Protein Albumin, Gelatine, Collagen, Zein, Casein oder Fibrinogen ist.

6. Verfahren nach Anspruch 5, worin das Protein humanes Serumalbumin ist.

7. Verfahren nach Anspruch 5, worin das Protein Ovalbumin ist.

8. Verfahren nach einem der vorhergehenden Ansprüche, worin das Protein dadurch modifiziert ist, daß es ein weiteres organisches Polymer daran gebunden aufweist.

9. Verfahren nach einem der vorhergehenden Ansprüche, welches die einzelnen Schritte umfaßt:

   (a) Mischen einer wäßrigen Lösung von mindestens einem Protein oder modifizierten Protein mit einer wäßrigen Lösung von mindestens einer α-Hydroxysäure oder eines Analogons oder Derivats davon,

   (b) Zugeben eines Koazervierungsmittels zu dem in Schritt (a) hergestellten Gemisch, was in der Bildung von Mikrosphären resultiert, in denen das Protein oder modifizierte Protein inkorporiert ist,

   (c) Verdampfen des Koazervierungsmittels und

   (d) Gewinnen der Mikrosphären aus der wäßrigen Lösung.

10. Verfahren nach Anspruch 9, worin das Koazervierungsmittel ausgewählt wird aus Aceton, Ethanol und Isopropanol.

11. Verfahren nach Anspruch 9 oder Anspruch 10, worin vor der Zugabe des Koazervierungsmittels ein pharmazeutischer Wirkstoff dem in Schritt (a) hergestellten Gemisch zugegeben wird.

12. Verfahren nach Anspruch 9 oder Anspruch 10, worin vor der Zugabe des Koazervierungsmittels ein durch ein Bioimagingverfahren nachweisbares Material dem in Schritt (a) hergestellten Gemisch zugegeben wird.

13. Verfahren nach einem der Ansprüche 1 bis 8, umfassend die einzelnen Schritte:

(a) Mischen einer wäßrigen Lösung von mindestens einem Protein oder modifizierten Protein mit einer wäßrigen Lösung von mindestens einer α-Hydroxysäure oder eines Analogons oder Derivats davon,

(b) Zugeben des in Schritt (a) hergestellten Gemisches zu einem mit Wasser nicht mischbaren Öl,

(c) Rühren des in Schritt (b) hergestellten Gemisches, um Mikrosphären zu bilden, und

(d) Gewinnen der Mikrosphären aus dem mit Wasser nicht mischbaren Öl.

14. Verfahren nach Anspruch 13, worin vor dem Gewinnen aus dem mit Wasser nicht mischbarem Öl den in Schritt (c) hergestellten Mikrosphären ein Lösungsmittel zugegeben wird, um eine Dispergierung zu unterstützen.

15. Verfahren nach Anspruch 14, worin das zur Unterstützung der Dispergierung zugegebene Lösungsmittel Aceton oder Ethylacetat ist.

16. Verfahren nach einem der Ansprüche 13 bis 15, worin die Mikrosphären vor dem Gewinnen aus dem mit Wasser nicht mischbarem Öl sonifiziert werden.

17. Verfahren nach einem der Ansprüche 13 bis 16, worin ein pharmazeutischer Wirkstoff dem in Schritt (a) hergestellten Gemisch vor dem Mischen mit dem mit Wasser nicht mischbaren Öl zugegeben wird.

18. Verfahren nach einem der Ansprüche 13 bis 16, worin ein durch ein Bioimagingverfahren nachweisbares Material dem in Schritt (a) hergestellten Gemisch vor dem Mischen mit dem mit Wasser nicht mischbaren Öl zugegeben wird.

19. Verfahren nach einem der Ansprüche 9 bis 18, welches den zusätzlichen Schritt umfaßt, daß an die Oberfläche der so gebildeten Mikrosphären ein Molekül gebunden wird, das von einem zellulären Rezeptor im menschlichen oder in einem Tierkörper erkannt wird und eine Affinität dafür aufweist.

20. Verfahren nach einem der Ansprüche 9 bis 18, welches den zusätzlichen Schritt umfaßt, daß an die Oberfläche der so gebildeten Mikrosphären ein antigenes Material gebunden wird.

21. Mikrosphäre, hergestellt durch das Verfahren nach einem der Ansprüche 9 bis 20.

22. Pharmazeutische Zusammensetzung, welche mehrere Mikrosphären, die durch das Verfahren nach Anspruch 11 oder Anspruch 17 hergestellt sind, und einen pharmazeutisch annehmbaren Träger oder Verdünnungsstoff umfaßt.

23. Impfstoff, welcher mehrere Mikrosphären, die durch das Verfahren nach Anspruch 19 hergestellt sind, und einen pharmazeutisch annehmbaren Träger oder Verdünnungsstoff umfaßt.

24. Bioimagingpräparat, welches mehrere Mikrosphären, die durch das Verfahren nach Anspruch 12 oder Anspruch 18 hergestellt sind, und einen pharmazeutisch annehmbaren Träger oder Verdünnungsstoff umfaßt.

25. Verfahren nach einem der Ansprüche 1 bis 8, welches die einzelnen Schritte umfaßt:

(a) Kühlen einer wäßrigen Lösung des Proteins oder modifizierten Proteins auf etwa 4 °C,

(b) Mischen der in Schritt (a) hergestellten gekühlten Lösung mit einer wäßrigen Lösung der α-Hydroxysäure oder eines Analogons oder Derivats davon,

(c) Ausbreiten der in Schritt (b) hergestellten Lösung als dünne Schicht über eine feste Oberfläche und

(d) Trocknen der dünnen Schicht, um einen Film zu bilden.

26. Verfahren nach Anspruch 25, worin die Lösungsschicht bei zwischen 50 und 70 °C getrocknet wird.

27. Film, hergestellt durch das Verfahren nach Anspruch 25 oder 26.

28. Verfahren zur Beschichtung eines Gegenstandes mit einer Protein- oder modifizierten Proteinschicht, welches das Eintauchen des Gegenstandes oder eines Teils davon in eine wäßrige Lösung umfaßt, die das Protein oder das modifizierte Protein und eine α-Hydroxysäure oder ein Analogon oder ein Derivat davon enthält.

29. Verfahren zur Behandlung eines mit einem Protein oder einem modifizierten Protein beschichteten Gegenstandes, welches das Eintauchen des Gegenstandes oder eines Teils davon in eine wäßrige Lösung umfaßt, die eine α-Hydroxysäure oder ein Analogon oder ein Derivat davon enthält.

30. Verfahren nach Anspruch 28 , welches die Merkmale von einem der Ansprüche 2 bis 8 aufweist.

Revendications

1. Procédé pour stabiliser une microsphère, un film ou un revêtement constitué d'au moins une protéine hydrosoluble ou d'une protéine modifiée hydrosoluble, procédé qui comprend la préparation de ladite microsphère, dudit film ou dudit revêtement de protéine en présence d'une solution aqueuse d'au moins un α-hydroxyacide ou d'un de ses dérivés ou analogues.

2. Procédé suivant la revendication 1, dans lequel l'α-hydroxyacide est l'acide glycolique, l'acide lactique, l'acide α-hydroxybutyrique ou un mélange de deux ou plus de deux de ces acides.

3. Procédé suivant la revendication 1, dans lequel le dérivé d'un α-hydroxyacide consiste en un α-hydroxyacide conjugué à du polyéthylène-glycol.

4. Procédé suivant la revendication 2 ou la revendication 3, dans lequel l'α-hydroxyacide consiste en l'acide lactique.

5. Procédé suivant l'une quelconque des revendications précédentes, dans lequel la protéine consiste en albumine, gélatine, collagène, zéine, caséine ou fibrinogène.

6. Procédé suivant la revendication 5, dans lequel la protéine consiste en sérum-albumine humaine.

7. Procédé suivant la revendication 5, dans lequel la protéine consiste en ovalbumine.

8. Procédé suivant l'une quelconque des revendications précédentes, dans lequel la protéine est modifiée par fixation d'un autre polymère organique à cette protéine.

9. Procédé suivant l'une quelconque des revendications précédentes, qui comprend les étapes particulières consistant :

   (a) à mélanger une solution aqueuse d'au moins une protéine ou protéine modifiée à une solution aqueuse d'au moins un α-hydroxyacide ou d'un de ses analogues ou dérivés,
   (b) à ajouter au mélange préparé dans l'étape (a) un agent de coacervation ayant pour résultat la formation de microsphères renfermant ladite protéine ou protéine modifiée,
   (c) à évaporer ledit agent de coacervation, et
   (d) à séparer les microsphères de la solution aqueuse.

10. Procédé suivant la revendication 9, dans lequel l'agent de coacervation est choisi entre l'acétone, l'éthanol et l'isopropanol.

11. Procédé suivant la revendication 9 ou la revendication 10, dans lequel, avant l'addition de l'agent de coacervation, un agent pharmaceutiquement actif est ajouté au mélange préparé dans l'étape (a).

12. Procédé suivant la revendication 9 ou la revendication 10, dans lequel, avant l'addition de l'agent de coacervation, une matière détectable par un mode opératoire de bio-imagerie est ajoutée au mélange préparé dans l'étape (a).

13. Procédé suivant l'une quelconque des revendications 1 à 8, comprenant les étapes particulières consistant :

(a) à mélanger une solution aqueuse d'au moins une protéine ou protéine modifiée à une solution aqueuse d'au moins un α-hydroxyacide ou un de ses analogues ou dérivés,
(b) à ajouter le mélange préparé dans l'étape (a) à une huile non miscible à l'eau,
(c) à agiter le mélange préparé dans l'étape (b) pour former des microsphères,
(d) à séparer les microsphères de l'huile non miscible à l'eau.

14. Procédé suivant la revendication 13, dans lequel, avant la séparation de l'huile non miscible à l'eau, un solvant est ajouté aux microsphères préparées dans l'étape (c) pour faciliter la dispersion.

15. Procédé suivant la revendication 14, dans lequel le solvant ajouté pour faciliter la dispersion est l'acétone ou l'acétate d'éthyle.

16. Procédé suivant l'une quelconque des revendications 13 à 15, dans lequel les microsphères sont traitées par ultrasons avant d'être séparées de l'huile non miscible à l'eau.

17. Procédé suivant l'une quelconque des revendications 13 à 16, dans lequel un agent pharmaceutiquement actif est ajouté au mélange préparé dans l'étape (a) avant l'étape de mélange à l'huile non miscible à l'eau.

18. Procédé suivant l'une quelconque des revendications 13 à 16, dans lequel une matière détectable par un mode opératoire de bio-imagerie est ajoutée au mélange préparé dans l'étape (a) avant l'étape de mélange à l'huile non miscible à l'eau.

19. Procédé suivant l'une quelconque des revendications 9 à 18, qui comprend l'étape supplémentaire consistant à fixer à la surface des microsphères ainsi formées une molécule reconnue par, et ayant une affinité pour, un récepteur cellulaire dans l'organisme de l'homme ou d'un animal.

20. Procédé suivant l'une quelconque des revendications 9 à 18, qui comprend l'étape supplémentaire consistant à fixer à la surface des microsphères ainsi formées une matière antigénique.

21. Microsphère préparée par le procédé suivant l'une quelconque des revendications 9 à 20.

22. Composition pharmaceutique qui comprend une pluralité de microsphères préparées par le procédé suivant la revendication 11 ou la revendication 17 et un support ou diluant pharmaceutiquement acceptable.

23. Vaccin qui comprend une pluralité de microsphères préparées par le procédé suivant la revendication 19 et un support ou diluant pharmaceutiquement acceptable.

24. Préparation pour bio-imagerie, qui comprend une pluralité de microsphères préparées par le procédé suivant la revendication 12 ou la revendication 18 et un support ou diluant pharmaceutiquement acceptable.

25. Procédé suivant l'une quelconque des revendications 1 à 8, qui comprend les étapes particulières consistant :

(a) à refroidir à approximativement 4°C une solution aqueuse de la protéine ou protéine modifiée,
(b) à mélanger la solution refroidie préparée dans l'étape (a) à une solution aqueuse de l'α-hydroxyacide ou d'un de ses analogues ou dérivés,
(c) à étaler la solution préparée dans l'étape (b) sous forme d'une couche mince sur une surface solide, et
(d) à sécher ladite couche mince pour former un film.

26. Procédé suivant la revendication 25, dans lequel la couche de solution est séchée à une température comprise dans l'intervalle de 50 à 70°C.

27. Film préparé par le procédé suivant la revendication 25 ou 26.

28. Procédé pour le revêtement d'un article avec une couche de protéine ou protéine modifiée, qui comprend l'immersion de cet article ou d'une partie de cet article dans une solution aqueuse comprenant ladite protéine ou protéine modifiée et un α-hydroxyacide ou un de ses analogues ou dérivés.

29. Procédé pour le traitement d'un article revêtu avec une protéine ou protéine modifiée, qui comprend l'immersion de cet article ou d'une partie de cet article dans une solution aqueuse comprenant un α-hydroxyacide ou un de ses analogues ou dérivés.

30. Procédé suivant la revendication 28, ayant les caractéristiques de l'une quelconque des revendications 2 à 8.

FIG. 1

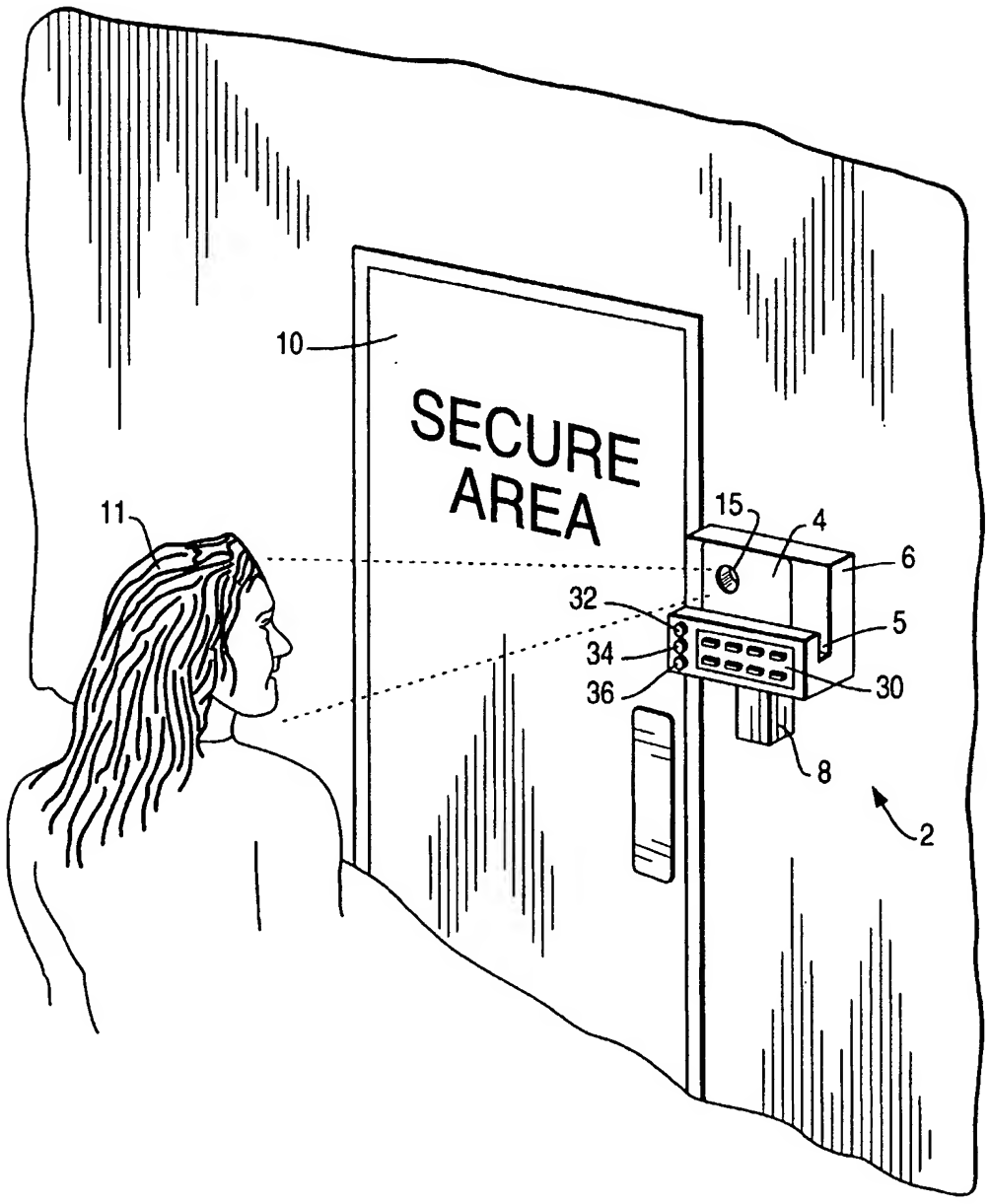


FIG. 2A

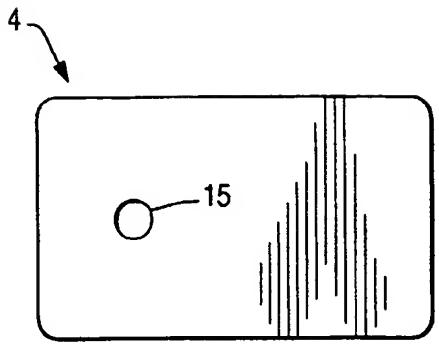


FIG. 2B

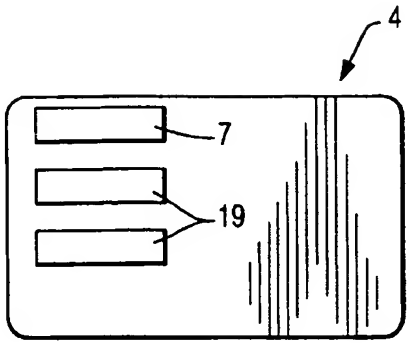


FIG. 3A

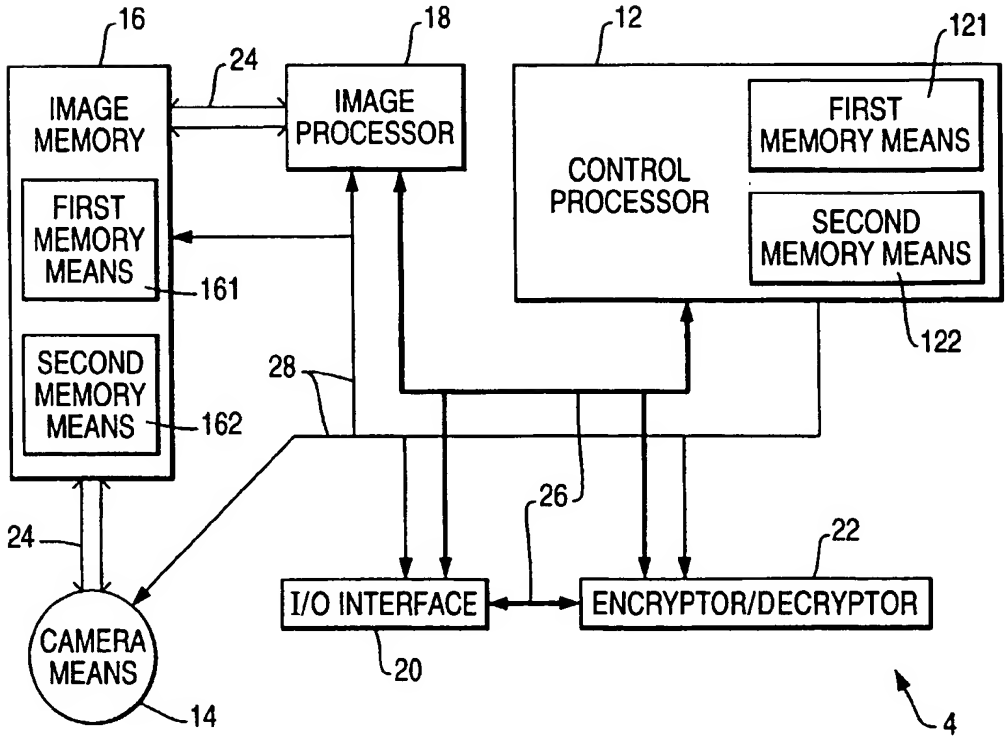
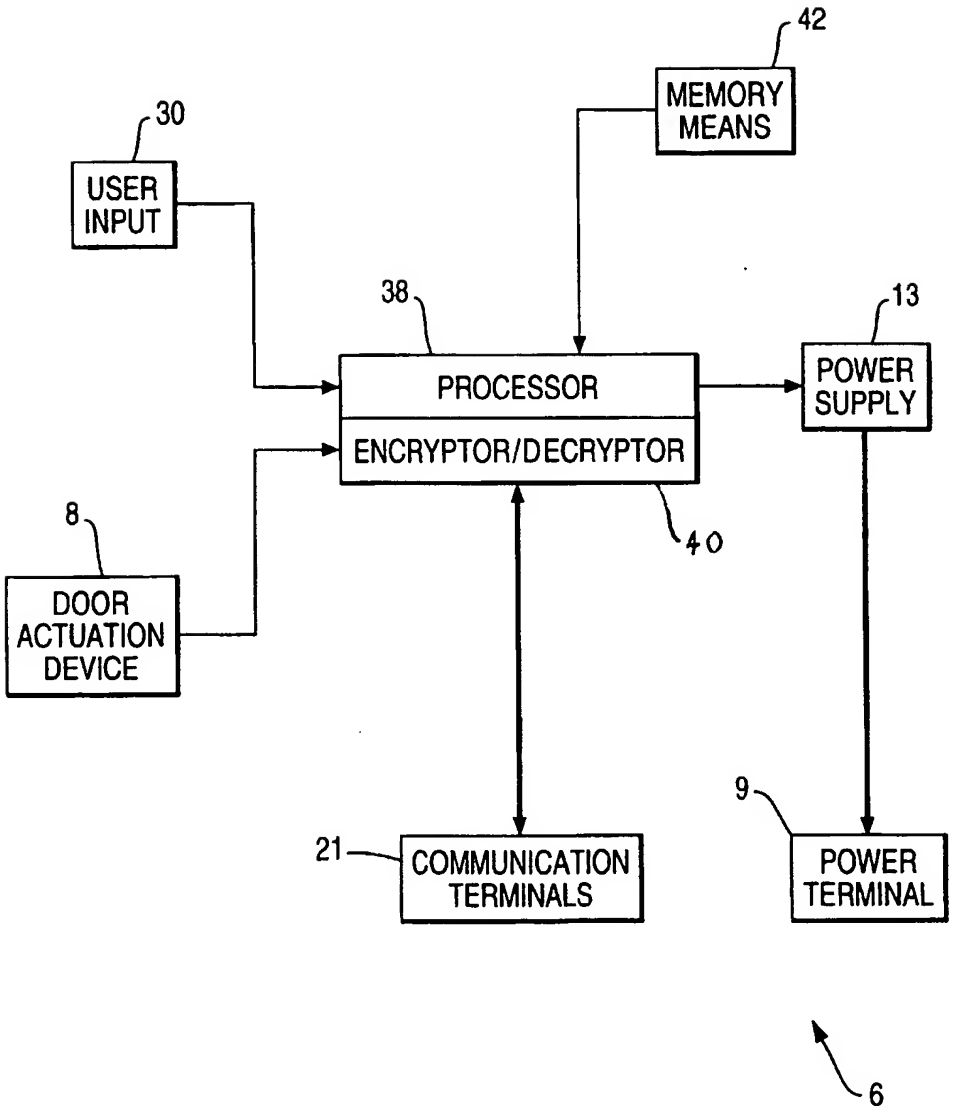


FIG. 3B


42
MEMORY MEANS
30
USER INPUT
38
PROCESSOR
ENCRYPTOR/DECRYPTOR
40
13
POWER SUPPLY
8
DOOR ACTUATION DEVICE
21
COMMUNICATION TERMINALS
9
POWER TERMINAL
6